

1 Claims

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- 3 1. A peptide display carrier package (PDCP), said
- 4 package comprising a recombinant polynucleotide-
- 5 chimeric protein complex wherein the chimeric
- 6 protein has a nucleotide binding portion and a
- 7 target peptide portion, wherein said recombinant
- 8 polynucleotide comprises a nucleotide sequence
- 9 motif which is specifically bound by said
- 10 nucleotide binding portion, and wherein at least
- 11 the chimeric protein-encoding portion of the
- 12 recombinant polynucleotide not bound by the
- 13 chimeric protein nucleotide binding portion is
- 14 protected by a binding moiety.
- 15
- 16 2. A peptide display carrier package (PDCP) as claimed
- 17 in Claim 1, wherein said chimeric protein-encoding
- 18 portion of the recombinant polynucleotide not bound
- 19 by the chimeric protein nucleotide binding portion
- 20 is protected by a non-sequence-specific protein.
- 21
- 22 3. A peptide display carrier package (PDCP) as claimed
- 23 in Claim 2, wherein said non-sequence-specific
- 24 protein is a viral coat protein.
- 25
- 26 4. A peptide display carrier package (PDCP) as claimed
- 27 in any one of Claims 1 to 3, wherein said target
- 28 peptide portion is displayed externally on the
- 29 package.
- 30
- 31 5. A peptide display carrier package (PDCP) as claimed
- 32 in any one of Claims 1 to 4 wherein said
- 33 recombinant polynucleotide includes a linker
- 34 sequence between the nucleotide sequence encoding
- 35 the nucleotide binding portion and the nucleotide
- 36 sequence encoding the target peptide portion.

1 6. A peptide display carrier package (PDCP) as claimed
2 in any one of Claims 1 to 5 wherein said
3 recombinant polynucleotide has two or more
4 nucleotide sequence motifs each of which can be
5 bound by the nucleotide binding portion of the
6 chimeric protein.

7
8 7. A peptide display carrier package (PDCP) as claimed
9 in any one of Claims 1 to 6 wherein said nucleotide
10 binding portion is a DNA binding domain of an
11 oestrogen or progesterone receptor.

12
13 8. A peptide display carrier package (PDCP) as claimed
14 in any one of Claims 1 to 7 wherein said
15 recombinant polynucleotide is bound to said
16 chimeric protein as single stranded DNA.

17
18 9. A peptide display carrier package (PDCP) as claimed
19 in any one of Claims 1 to 8 wherein said target
20 peptide portion is located at the N and/or C
21 terminal of the chimeric protein.

22
23 10. A peptide display carrier package (PDCP) as claimed
24 in any one of Claims 1 to 9 which is produced in a
25 host cell transformed with said recombinant
26 polynucleotide and extruded therefrom without lysis
27 of the host cell.

28
29 11. A recombinant polynucleotide comprising a
30 nucleotide sequence encoding a chimeric protein
31 having a nucleotide binding portion operably linked
32 to a target peptide portion, wherein said
33 polynucleotide includes a specific nucleotide
34 sequence motif which is bound by the nucleotide
35 binding portion of said chimeric protein and
36 further encoding a non-sequence-specific nucleotide

1 binding protein.

2

3 12. A recombinant polynucleotide as claimed in Claim 11
4 wherein said non-sequence-specific nucleotide
5 binding protein is a viral coat protein.
6

7 13. A recombinant polynucleotide as claimed in either
8 one of Claims 11 and 12 which includes a linker
9 sequence between the nucleotide sequence encoding
10 the nucleotide binding portion and the nucleotide
11 sequence encoding the target peptide portion.
12

13 14. A recombinant polynucleotide as claimed in any one
14 of Claims 11 to 13 which has two or more nucleotide
15 sequence motifs each of which can be bound by the
16 nucleotide binding portion of the chimeric protein.
17

18 15. A recombinant polynucleotide as claimed in any one
19 of Claims 11 to 14 wherein said nucleotide binding
20 portion is a DNA binding domain of an oestrogen or
21 progesterone receptor.
22

23 16. A recombinant polynucleotide as claimed in any one
24 of Claims 11 to 15 wherein said recombinant
25 polynucleotide is bound to said chimeric protein as
26 single stranded DNA.
27

28 17. A genetic construct or set of genetic constructs
29 which collectively comprises a polynucleotide
30 having a sequence which includes:

- 31 i) a sequence encoding a nucleotide binding
32 portion able to recognise and bind to a
33 specific sequence motif;
34 ii) the sequence motif recognised and bound by the
35 nucleotide binding portion encoded by (i);
36 iii) a restriction enzyme site which permits

1 insertion of a polynucleotide, said site being
2 designed to operably link said polynucleotide
3 to the sequence encoding the nucleotide
4 binding portion so that expression of the
5 operably linked polynucleotide sequences
6 yields a chimeric protein; and

7 iv) a sequence encoding a nucleotide binding
8 protein which binds non-specifically to naked
9 polynucleotide.

10
11 18. A genetic construct or set of genetic constructs as
12 claimed in Claim 17 wherein a linker sequence is
13 located between the nucleotide sequence encoding
14 the nucleotide binding portion and the site for
15 insertion of the polynucleotide.

16
17 19. A genetic construct or set of genetic constructs as
18 claimed in either one of Claims 17 and 18 which
19 includes a vector pDM12, pDM14 or pDM16, deposited
20 at NCIMB under Nos 40970, 40971 and 40972
21 respectively.

22
23 20. A method of constructing a genetic library, said
24 method comprising:

25
26 a) constructing multiple copies of a recombinant
27 vector comprising a polynucleotide sequence
28 which encodes a nucleotide binding portion
29 able to recognise and bind to a specific
30 sequence motif;

31
32 b) operably linking each said vector to a
33 polynucleotide encoding a target polypeptide,
34 such that expression of said operably linked
35 vector results in expression of a chimeric
36 protein comprising said target peptide and

1 said nucleotide binding portions; wherein said
2 multiple copies of said operably linked
3 vectors collectively express a library of
4 target peptide portions;

5
6 c) transforming host cells with the vectors of
7 step b);

8
9 d) culturing the host cells of step c) under
10 conditions suitable for expression of said
11 chimeric protein;

12
13 e) providing a recombinant polynucleotide
14 comprising the nucleotide sequence motif
15 specifically recognised by the nucleotide
16 binding portion and exposing this
17 polynucleotide to the chimeric protein of step
18 d) to yield a polynucleotide-chimeric protein
19 complex; and

20
21 f) causing production of a non-sequence-specific
22 moiety able to bind to the non-protected
23 portion of the polynucleotide encoding the
24 chimeric protein to form a peptide display
25 carrier package.

26
27 21. A method of screening a genetic library, said
28 method comprising:

29
30 a) exposing the polynucleotide members of said
31 library to multiple copies of a genetic
32 construct comprising a nucleotide sequence
33 encoding a nucleotide binding portion able to
34 recognise and bind to a specific sequence
35 motif, under conditions suitable for the
36 polynucleotides of said library each to be

1 individually ligated into one copy of said
2 genetic construct, to create a library of
3 recombinant polynucleotides;
4

5 b) exposing said recombinant polynucleotides to a
6 population of host cells, under conditions
7 suitable for transformation of said host cells
8 by said recombinant polynucleotides;
9

10 c) selecting for transformed host cells;
11

12 d) exposing said transformed host cells to
13 conditions suitable for expression of said
14 recombinant polynucleotide to yield a chimeric
15 protein; and
16

17 e) providing a recombinant polynucleotide
18 comprising the nucleotide sequence motif
19 specifically recognised by the nucleotide
20 binding portion and exposing this
21 polynucleotide to the chimeric protein of step
22 d) to yield a polynucleotide-chimeric protein
23 complex;
24

25 f) protecting any exposed portions of the
26 polynucleotide in the complex of step e) to
27 form a peptide display carrier package; and
28

29 g) screening said peptide display carrier package
30 to select only those packages displaying a
31 target peptide portion having the
32 characteristics required.
33

34 22. A method as claimed in Claim 21 wherein the peptide
35 display package carrier is extruded from the host
36 cell without lysis thereof.

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